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Journey of organ on a chip technology and its role in future healthcare scenario

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ABSTRACT

Organ on a chip refers to microengineered biomimetic system which reflects structural and functional characteristics of human tissue. It involves biomaterial technology, cell biology and engineering combined together in a miniaturized platform. Several models using different organs such as lungs on a chip, liver on a chip, kidney on a chip, heart on a chip, intestine on a chip and skin on a chip have been successfully developed. Food and Drug administration (FDA) has also shown confidence in this technology and has partnered with industries/institutes which are working with this technology. In this review, the concepts and applications of Organ on a chip model in different scientific domains including disease model development, drug screening, toxicology, pathogenesis study, efficacy testing and virology is discussed. It is envisaged that amalgamation of various organs on chip modules into a unified body on chip device is of utmost importance for diagnosis and treatment, especially considering the complications due to the ongoing COVID-19 pandemic. It is expected that the market demand for developing organ on chip devices to skyrocket in the near future.

Introduction

Organ on a chip (OOAC) is a novel *in-vitro* micro-scale biomimetic platform that helps in reproducing physiological environment of human organs. This technology involves cell biology, engineering and material sciences to simulate *in-vivo* tissues [1]. *In-vivo* experiments on animals and humans are common to study physiological functions of the body but several alternative methods, including 2D and 3D *in-vitro* models and computational approaches, have also been explored in last two decades.

Since 2011, when US president announced the start of project on "human on chip" by the US research agencies, researchers across the globe became curious to understand and work on the possibilities of this concept. It has advantage of miniaturization, integration, low consumption and accurate control of parameters such as concentration gradient, fluid shear stress, organ–organ interaction, tissue–tissue interface [2]. Organ on a chip model has shown immense usefulness in drug discovery process. It can be used for hit-to-lead optimization, toxicological studies, physiological studies, pharmacokinetic studies and phenotyping screening [3].

The induced pluripotent stem cell (iPSC) technology which was introduced in 2006 became a common and effective source for supplying human cells for different organs such as brain, heart, spinal cord, kidney etc.

The origin of 2D cell culture is traced to Ross Harrison, who studied neural tissue growth as early as in 1907. Mid 1950s was era of 2D cell culture advances when establishment of "L" cell line and human HeLa cell line of a cancer patient were done, both continue to drive cell culture research even today [4]. The 2D cell culture has been the mainstay for scientists to understand human physiology, culture stem cells, study diseases, cell-cell interaction, tissue imaging, drug discovery, toxicology and drug metabolism since 1900s [5]. However, it has its own disadvantages such as it is unable to mimic the actual in vivo scenario where in cell-to-cell contact is further fortified by extra-cellular matrix. The cells are not monolayers but intricately arranged as tissues wherein cell-cell interaction also leads to mechanical force being exerted by one cell on another. Thus, cells in 2D do not depict the true physiology of the organ. Cells in 2D cultures experience no gravity. Further, 2D culture does not give layers of cells to study drug efficacy and penetration into cells. Thus, it failed to provide accurate micro-environment for drug discovery [6]. Adding to this, it is known that many drug discovery

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studies have faced a set back at phase III level of development either due to toxicity or lack of efficacy and thus affected the economics of drug discovery process adversely [7, 8]. The 2D cultured cells have altered cell morphology, show cell flattening leading to cytoskeletal and changes in shape of nucleus [9,10]. The cells in 2D culture get exposed to equal amounts of media, oxygen being in suspension while to model cancers, a mass of cells has to be generated where oxygen tension and nutrient concentrations are much less in the inner layers of the mass than outer layers [11]. The 2D cultured cells are always proliferating and do not depict the state of tissues where all cells are at different stages of growth and cell cycle. Scaling up of 2D cell cultures has been difficult. Last but not the least, an important drawback is that the cells in 2D are in stress [12].

Late 20th century saw a surge in development of 3D cell culture technologies. This recent advent of 3D culturing design and technologies allowed various improvements in cell research and led to a more accurate depiction of cellular processes. However, a new technology brings newer challenges. The advantages of 2D cultures are that the scientific community is trained on 2D cell culture, imaging protocols are well developed, it is easier to image cells in x-y plane than to do a 3D imaging, comparative literature on 2D cells is available in plenty and it is inexpensive to do a 2D culture.3D technology offers many advantages such as it mimics biological environment more, allows accurate research on cell–cell interaction and cell-ECM (extra cellular matrix) interactions. Various studies in drug discovery and cancer conducted on 3D cell culture achieved better accuracy [13, 14, 12]. 3D cell culture also helped in subverting research on animal models and thereby making the research more humane. The gene expression studies correlate better with 3D models of cells [15, 16, 17]. Fig. 1 shows graphical representation of 2D cell culture, 3D cell culture and organ on a chip model. There are numerous ways to culture cells in 3D which can be classified into scaffold based or scaffold free. The scaffold based models include polymeric hard material-based support, hydrogel-based support, hydrophilic glass fibre, and organoids each with unique applications, advantages and disadvantages. The non-scaffold based methods include hanging drop microplates, magnetic levitation, and spheroid microplates with ultra-low attachment coating; each has its unique advantage and disadvantages [18].

However, 3D culture have limitations such as the matrices/scaffolds used may have unwanted viral, human derived hormonal components making them less adaptable to clinical set up, detachment of 3D cultures is difficult [4, 19]. They suffer from batch to batch variability, isolation of nucleic acids/ proteins from 3D cell culture is much more difficult [19]. Further, cytometry analysis requiring single cells are optimized for 2D cell culture suspensions. The most recent development post 3D cell culture is its amalgamation with microfluidics to build cell based sensors on a chip (where 2D cultured static cells were used) now advanced to organ on a chip [20]. Table 1 summarizes the limitations and advantages of 2D and 3D cell culture.

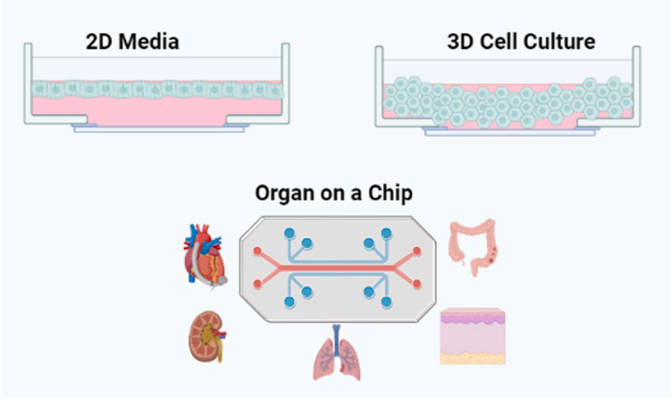

Fig. 1. Graphical representation of 2D cell culture, 3D cell culture and Organ on a chip model.

The first-generation microfluidic biological devices ideally called as 'cells on a chip'. Microfluidics involves study and manipulation of fluids in microliter scale (μL) confined within channels with dimensions of few tens to hundreds of micrometres. The earliest microfluidics devices allowed improved understanding of micro analytics, molecular analysis (such as microarrays) and also had applications in defence [21]. The amalgamation of microfluidics and 3D cell cultures adds another dimension to cell biology research leading to better mimicking of *in-vivo* cell environment. It allow studies of biological organs with minute volumes of fluid. They add multiple dimensions to cell research for they can be easily miniaturized, are easy to use, highly sensitive and robust, and adaptable towards a high throughput design. 3D Bioprinting has allowed fast paced development of organ on a chip microfluidic devices [22, 23]. Some applications of organ on a chip models are described in Table 2.

Types of organ on a chip models

In recent years, researchers developed different models for different organs on a chip, for example; kidney on a chip [40], lung on a chip [41], heart on a chip [42], skin on a chip [43], pancreas on a chip [44], brain on a chip and blood brain barrier on a chip [45].

Microfluidic technologies that enabled developing organ on a chip models overcame some of the current limitations such as:

a Organ's exposure to fluids in motion which is often directional.
b Nutrients flowing in and the waste flowing out.
c A gradient flow can be established leading to dosing studies becoming more amenable.
d Multiple cell types can be layered thus mimicking organs.

Table 1
Comparison of features of 2D cell culture with that of 3D.

Features/ Characteristics	2D cell culture	3D cell culture
Cell Morphology	Flattened/Elongated Only 2D expansion is allowed	Native cell phenotype is preserved Cells grow in 3 dimensions
Exposure to cell medium	Cells receive equal exposure to media Cells are maintained in same phase of growth gases diffuse into and out cells more evenly	Cells in center of 3 mass are less exposed to media Due to differential exposure of media, cells are in different phases of growth Diffusion of gases is uneven in inner layers versus outer layer of cells
Cell differentiation	Cells in 2D do not differentiate well	Cells in 3D culture differentiate well
Cell junctions	No real mimic of cell-cell junctions are formed in 2D cell cultures	Cell-cell junctions are observed and mimic real cell junctions
Drug sensitivity	Cells are often unrealistically susceptible to drugs	Cells are often resistant to drugs compared to 2D and results are comparable to in vivo models
Cell proliferation	Very rapid, unnatural	Realistic, mimics natural conditions
Imaging and analysis	Existing procedures are standardized for 2D cells Imaging analysis is standard and easier for 2D cells	Procedures are not standardized for 3D cells Imaging and analysis is difficult for 3D cells
Gene expression	Gene expression does not mimic that *in-vivo* models	Gene expression more accurately depicts that *in- vivo* models
Cost	Low	High/expensive

Table 2
Summary of applications of Organ on a Chip technology.

Organ on a Chip	Applications/ Model	References
Lung on a Chip	Model pulmonary edema, *in-vivo* environment for human airways, model for viral infection	[24, 25],
Brain on a Chip	Blood Brain Barrier functioning, Neural Network	[26, 27, 28, 29]
Heart on a Chip	Electrical stimulation, cardiac electrophysiology and different heart diseases	[30, 31, 32]
Liver on a Chip	Liver specific Protein Synthesis,	[33, 34]
Kidney on a chip	Drug induced nephrotoxicity, Glomerular filtration	[35, 36]
Skin on a Chip	Dermal diffusion testing, toxicology studies, efficacy testing, wound healing, inflammation, repair, ageing and shear stress studies	[37, 38]
Gut on a Chip	Drug pharmacokinetics, host-gut microbiota cross talk, and nutrition metabolism	[39]

e Studies are done on human derived cells, therefore physiologically much more relevant.
f Organ engineering can be done (with precise deletion/mutation) and studied in isolation unlike if it were an animal where other mutations would interfere.

The greatest revolution in research that came about by organ on a chip microfluidic devices is in areas of high throughput drug screening [46, 47], single cell analysis [48], cell–cell interaction, cell-ECM studies [49] cell co-culture [50], neuronal models [51, 52] and fluid gradient involving studies such as bacterial chemotaxis [53], drug screening, precision medicine (shown in Fig. 2), cancer cell migration and axon growth [54, 55, 56, 57].

Lung on chip

Pulmonary diseases are reported to be the fifth most common cause of death globally. Several new interventions are tried to facilitate the treatment of pulmonary diseases.

Huh et al. from Wyss Institute of Harvard University were the pioneers in developing lung on a chip model [58]. In 2015, Huh and co-workers prepared an architecture and dynamic microenvironment surrogate to alveolar–capillary unit of the living human lung (as shown in theFig. 3). The system was then used to conduct nano-toxicological study in which production of intracellular reactive oxygen species (ROS) in response to alveolar exposure to nanoparticles was examined. It was also used to model pulmonary edema in human lungs [24].

Cigarette smoking is the major cause for clinical exacerbations with patients with asthma and chronic obstructive pulmonary disease (COPD) Benam et al. developed a chip which breathes smoke in and out to study impact of smoking on lungs. They confirmed that experimental results obtained by using the lung-on-a-chip model were close to those from animal experiments. The team studied the up regulation and down regulation of genes due to smoking and also discovered novel biomarkers using this model [59]. Shrestha et al. fabricated simple open well lung on a chip model that could simulate the *in-vivo* environment of airway. They tested the cell viability, mucus secretion, cell permeability and P-gp expression on cell surface and found promising results for toxicology tests, permeability assays, pulmonary drug delivery studies. The effect of cigarette smoke on interlukins (IL-6 and IL8) followed by treatment with budesonide was studied using Calu-3 cells on lung-on a chip model(Jesus Shrestha, 2019).

Human airway on a chip was used for evaluating repurposing of the drugs for COVID-19. The model was developed for viral infection, cytokine production and circulating immune cells using human bronchial epithelium and pulmonary endothelium. Drugs such as nafamostat, olsetamivir, amodiaquine and hydroxychloroquine werestudied against pseudotypedSevere Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and influenza A [25].

Brain on chip

Engineering full structure and function of brain is difficult, yet some specific parts/functions such as structure of spinal cord, unidirectional neural network, myelination process and BBB (Blood Brain Barrier) have been successfully recapitulated. Brain on a Chip designs are broadly classified into 4 groups and are summarized in Table 3: microfluidic models, compartmentalized models, hydrogel-based models and spheroid models. Each type of design has advantages in a particular application. Mold lithography, contact printing, hydrogel casting and 3D printing are the most common manufacturing processes for brain on chips [60].

Three-dimensional neurospheroids based microchip with constant flow of fluid was developed for in-vitro study of Alzheimer's disease. The toxic effects of amyloid β were studied by Park et al. for understanding destruction of neural networks. The destruction of neural networks was found to be significantly more as compared to when studied under static conditions [64]. Another model for neural differentiation and maturation was developed for analysis of complex cell and tissue behaviour. Through this brain on a chip model the migration of human neural progenitors in response to CXCL12, a key chemokine present in brain was studied [65]. Interconnected brain on a chip systems have also been made to study interaction of various cells of neurovascular unit as well as to study the interactions between brain and other organs [66, 67].

Blood brain barrier (BBB) maintains the homeostasis of brain by regulating traffic of molecules between blood and CNS. Any disturbance in this barrier results in several neurological disorders. Patient specific stem cells have been combined with microfluidic platform to form personalized human BBB-chip that simulates BBB and can predict the inter-individual variability. These chips were used to study the alterations in BBB function in diseased conditions. Huntington's disease (HD)

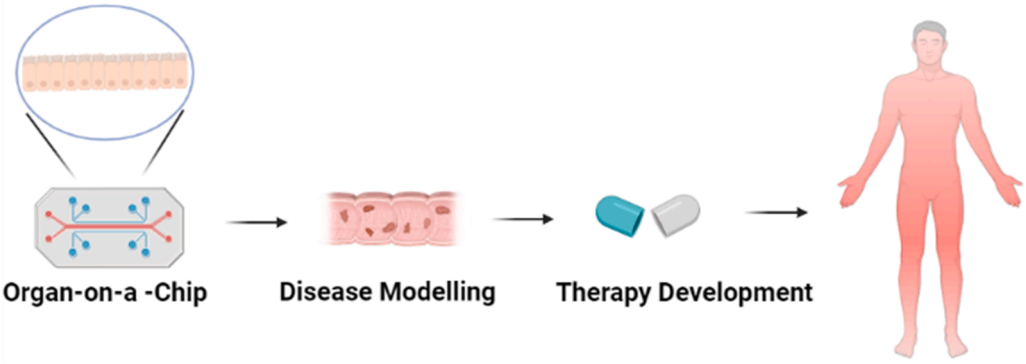

Fig. 2. Graphic representing organ on a chip development for disease modeling and therapy development for precision modeling.

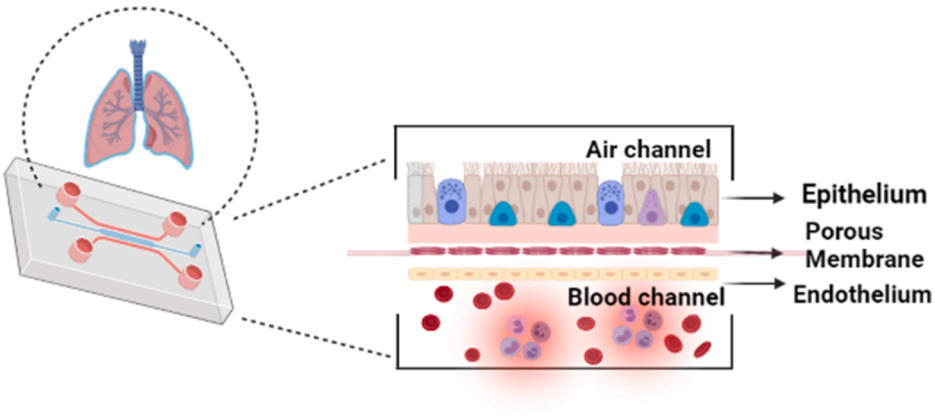

Fig. 3. Schematic of lung on chip portraying two sections namely - air channel and blood channel which mimics the human lung [24].

Table 3
Different Brain on a chip models and their applications.

Models	Applications	References
Microfluidic models	Study of tight junctions, and BBB functions	[61]
Compartmentalized models	Study of diseases development and behavior associated with neural networks	[27, 28, 29]
Hydrogel based models	These models biomimic in-vivo microenvironment. They allow cells to interact, migrate, and propagate in 3D. They also exhibit transport properties that mimic in vivo conditions	[62]
Spheroid models	Disease modeling and drug discovery	[63]

was studied with respect to the alteration in the barrier functions using induced brain microvascular endothelial cells. The study was based on variability in the permeability of fluorescently labeled dextrans (different molecular weights) across iPSC based BBB chips derived from healthy as well as HD patient. No significant variation in the permeability for the healthy humans but significant increase in some dextrans in HD patient was observed [26].

Sances *et al.* were able to develop spinal cord on a chip model using induced pluripotent stem cell (iPSC)-derived ventral spinal neurons which were co-cultured with brain endothelial cells. The uniqueness of this model is that it recapitulated the vascular-neural interaction and gene specific activation that enhanced the neuronal function and *in-vivo* like signatures. This model is proposed to provide platform to study, functional, pathological and disease mechanisms for therapeutic discoveries [68].

Heart on chip

Cardiovascular diseases (CVD) are leading cause of death in several countries. The major challenges of cardiovascular drug development are: (a) CVD animal models are poor predictors of human responses (b) adverse effects are organism dependent and (c) Lengthy and costly process. In a study analyzing the drug pipeline of AstraZeneca between 2005 and 2010, it was found that 82% of projects were closed in preclinical phase because of safety concerns. Cardiovascular related failures were around 17%, highest amongst all other organs analyzed [30].

Heart on a chip is used to study different functions of heart such as electrical stimulation, cardiac electrophysiology and different heart diseases. This model is also anticipated to be used in understanding particulate matter toxicity in human body [59]. Heart on a chip is useful in simulation of hypoxia, tachycardia model of arrythmogenesis and mechanical stimulation experienced by cardiac cells. It has also been used in combination with stem cells for therapeutic testing or precision medicine [31].

Kim and colleagues developed a multichannel microfluidic device containing fibrin matrix (to enable cell growth) which was used to study vasculogenesis and angiogenesis. They were able to mimic the perfusable vascular network and the barrier function. The vascular networks showed higher production of nitric oxide with dynamic environment as compared with the static conditions [32].

Microfluidic chip based on human induced pluripotent stem cells (iPSCs) – derived 3D cardiac microtissues was used to study contractile function with the help of MUSCLEMOTION software. Heart microchip device was also used to study the effect of isoproterenol, a β-adrenoceptor agonist, on the beating rate of 3D cardiac microtissues. The entire particle displacement distance was correlated with the beating rate with respect to the increased dose of isoproterenol [69]. Ischemia on a chip model was developed integrating intra and extracellular compartments with bioelectronic device. The model was able to mimic temporary coronary occlusion and reversibly activated hypoxia-related transduction pathways in HL-1 cardiac model cells [70].

Beating heart on a chip was developed with highly functional microengineered cardiac tissues which can be used to predict hypertrophic changes in cardiac cells. The device has ability to generate cardiac microtissues with increased mechanical and electrical coupling amongst neighboring cells. The model showed positive chronotropic effect with isoprenaline, hence, shows its possible use in drug discovery and toxicity studies [26].

Liver on chip

Most of the drugs fail in preclinical trials due to their hepatotoxic effects [34]. Liver on a chip model has a potential to be an economic and rapid alternative to animal studies [71].

Liver on a chip has offered advantage to study organogenesis, disease mechanisms, liver disease models, drug development and personalized medicines [72]. A microchip containing hepatocytes co-cultured with Kuppfer cells was developed to study the role of liver cells in hepatitis B virus (HBV). The model was compared with already existing 2D and 3D hepatic spheroids and results showed that due to constant recirculating nutrients and oxygenated media, the chip-based model can be used for long term study (at least 40days post seeding). The model can be used for host/pathogen interactions, biomarkers development, treatment responses and other therapeutic interventions [73].

A study done by Tostoes et al. showed that human liver-specific protein synthesis, CYP450 activity, and phase II and III drug-metabolizing enzyme gene expression activity was maintained in a perfusion bioreactor system for two to four weeks [33].The liver on a chip models have also been used to study hepatotoxicity of non-steroidal anti-inflammatory drugs (NSAIDS). Primary rat hepatocytes were used for toxicity testing of diclofenac and acetaminophen in a perfusion incubator liver chip [74].Layers of Caco2 (for gut) and HepG2 (for liver) cells were used to study first pass metabolism of apigenin using

microfluidic chip. CYP450 activity and absorptive property of Caco2 and HepG2 cells was also studied [75]. Using the same cells, Lee et al. predicted the first pass metabolism of paracetamol [76].

A spheroid based microfluidic chip with rat primary hepatocytes and hepatic stellate cells (HSCs) was developed as a model for alcohol liver injury. The role of HSCs was studied in the recovery of liver with alcohol liver disease [77].

Presently, there is no specific treatment of Non-alcoholicsteatohepatitis (NASH) which is mainly due to the absence of models that can recapitulate liver cellular microenvironment and the complexities of NASH. Freag et al. developed NASH on a chip to study disease pathogenesis and development of anti-NASH drugs [78].

Kidney on a chip

Kidney cells are at much less shear rate as compared to endothelial cells or lung cells. The initial design of Kidney on a chip had two compartments, the first compartment represented urinary lumen and has a fluid flow and the other chamber mimics interstitial space. The device utilized rat tubular cells. [79]

In 2013, Jang et al. reported development of a kidney-on-a-chip microfluidic model. The model could successfully exhibit number of primary cilia, expression of sodium-potassium ATPase and aquaporin 1, albumin uptake, and glucose reabsorption [80]. Drug induced nephrotoxicity studies were successfully done using microchips [35]. Membrane permeability and drug induced toxicity was studied for cisplatin, gentamycin and cyclosporine A [81]. In one of the study, glomerular function was studied with mature human podocytes derived from human induced pluripotent stem (hiPS) cells that could mimic the adriamycin induced albuminaria [36]. Kidney on a chip has also been utilized in multiple organ on a chip models (described below).

Podocyte is a glomerular visceral epithelial cell which acts as a size and charge selective barrier to plasma proteins and injury to podocytes can cause proteinurea. Podocytes on a chip has been tried by researchers but the system is challenging as sophisticated culturing is required. [82].

Skin on a chip

The source of skin cell lines are induced pluripotent stem cells (iPSC) or commercially available reconstructed skin tissues (EpidermFT, Epiderm, EpiSkin, StratiCELL) [83]. Skin on a chip models have been used for dermal diffusion testing, toxicology studies, efficacy testing, wound healing, inflammation, repair, ageing and shear stress studies [37]. Kim et al. studied the anti-ageing effects of curcumin and coenzyme Q10 using pumpless skin on a chip model [84, 85].

Wufuer et al. developed skin on a chip model and studied structural and functional features of the skin. Skin inflammation and edema was simulated through the model using TNF-α and the cytokine levels were studied. The anti-inflammatory response of steroidal drug, dexamethasone, was studied using this model as shown in Fig. 4 [38].

Sriram et al. [86] studied the barrier function and epidermal morphogenesis through fibrin based dermal matrix using organ on a chip model [86]. The diffusion studies through dermal layer including the role of efflux transporters, penetration and drug-drug interactions have been studied by many researchers [87, 88].

Gut on a chip

Artificial gut has been engineered on a chip with controlled microenvironment containing different types of human cells such as intestinal epithelial, endothelial and immune cells. The intestinal villi microstructures have also been reproduced to simulate *in-vivo* microenvironment of intestine [89]. Different types of cells used for this model are Caco2, human umbilical vein endothelial cells, intestinal organoids, human intestinal microvascular endothelial cells, human lymphatic microvascular endothelial cells and peripheral blood mononuclear cells. Gut on a chip has been used to study drug pharmacokinetics, host-gut microbiota cross talk, and nutrition metabolism [39].

Microfluidic microhole trapping array has been used to study the cellular permeability of propranolol, naproxen, furosemide, antipyrine, verapamil, atenolol, piroxicam, hydrochlorothiazide, cimetidine, and carbamazepine [90].

Gut on a chip microfluidic device-based model was developed for studying the Coxsackie B1 virus infection and interaction between host and infective virus [91]. Grassat and group studied the impact of intestinal mechanical forces on *Shigella* infection [92].

Multiple organs on a chip

Intestine–kidney chip was successfully developed to study the absorption and nephrotoxicity of digoxin in combination with cholestyramine and verapamil [93]. An intestine-liver microchip was developed with three sections comprises of intestinal cells, liver cells and breast cancer cells. Caco2 cells (for intestine) and HepG2 cells (for liver) were

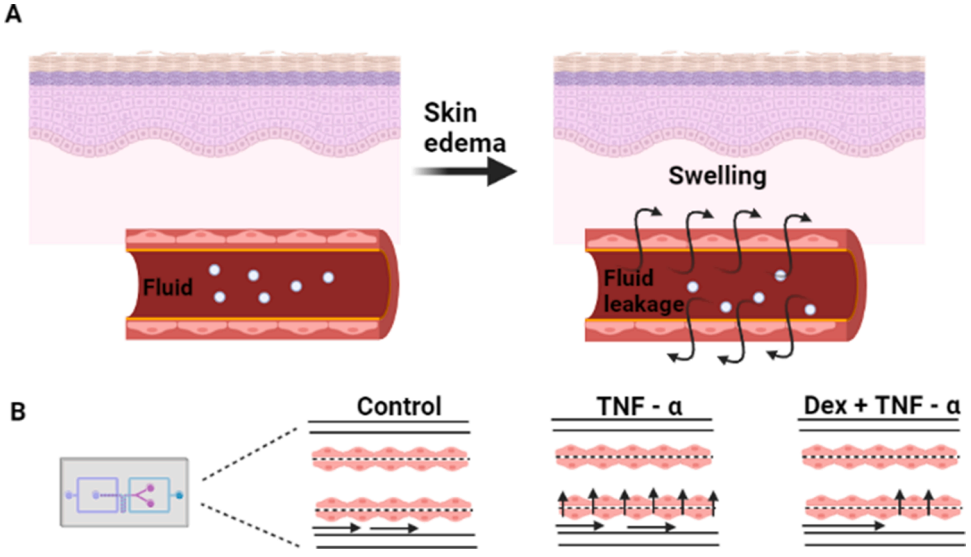

Fig. 4. (A) Schematic representation of the human skin edema model, (B) Inflammation induced by TNF-α damages tight junctions, resulting in vascular leakage and efficacy testing with dexamethasone [38].

used to study absorption and hepatic metabolism of cyclophosphamide, epirubicin, 17-β estradiol, and soy isoflavone. Drugs/substances after passing through the HepG2 cells were further analyzed for the anti-cancer activity using MCF-7 cells (mimics human breast carcinoma). The bioassay was performed with ease with lower consumption of cells as shown in Fig. 5 [94].

Three organs small intestine, liver and lung were simulated on a microfluidic chip. The organ-to-organ network was prepared through microporous membrane and microchannels. Caco-2, HepG2, and A549 cell were used to represent the small intestine, liver, and lung respectively. Due to perfusion environment and high oxygen permeability of polydimethylsiloxanes (PDMS), the device was able to co-culture three types of cells for at least more than 3 days. This model was used to study the pharmacokinetics of three anticancer drugs (epirubicine, irintecan and cyclophosphamide) and the results suggested that the device can replicate the bioactivity of anticancer drugs on target cells [94].

Micro-engineered chip devices

Most common methods, type of microarchitectures and materials used for fabrication of microchips are mentioned in Table 4 [95, 96, 97].

Most of the organs have a multimodular structure and are comprised of cells that play a characteristic function in the body such as gas exchange in the lung alveoli, metabolism in the liver and absorption in the villi of the gut [98].

Most popular source of biological tissues for different organ on a chip models are embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs) [1]. Different types of cells used for simulating the organs on a chip are summarized in Table 5.

Organ on a chip system: regulatory authorities and market size

Although organ on a chip technology is still in its infancy stage, regulatory bodies and top pharma companies are increasingly showing interest in this technology. Till now organ on a chip technology has not been specifically classified by any major regulatory body such as the United States Food and Drug Administration (U.S. FDA), the European Medicines Agency, and/or the Medicines and Healthcare Products Regulatory Agency in the United Kingdom. However, a survey showed that most developers of organ on a chip technology follow one of the following three guidelines: ISO 9001:2015, FDA 21 CFR Part 58, and FDA FD&C Act Section 507 [101].

National Center for Advancing Translational Science (NCATS) USA, the USFDA, and the US Defense Advanced Research Projects Agency (DARPA) collaborated to develop organ on a chip for screening drug safety and effectiveness before approval for first in human studies [102].

In 2017, the US FDA announced an agreement with a company named Emulate Inc, (Boston, MA) for evaluation of company's organs-on-chips technology at the agency's Center for Food Safety and Applied Nutrition (CFSAN). The aim of this project was to understand the usefulness of this technology for predicting the harmful effects of certain potential chemicals on the human body [102].FDA awards contract to Harvard University's Wyss Institute for Biologically Inspired Engineering for developing countermeasures to treat acute radiation syndrome (ARS). ARS illness affects combination of organs on exposure to high dose of radiation.

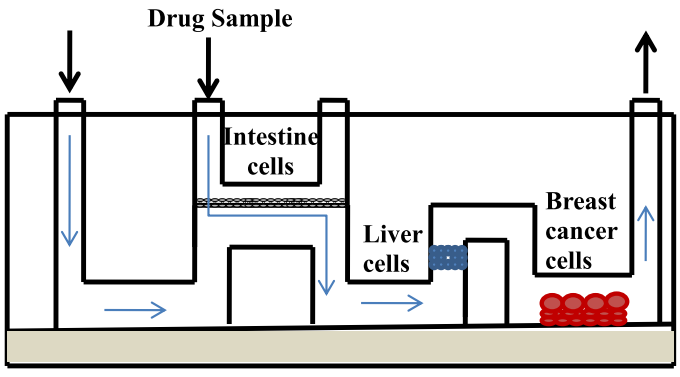

Fig. 5. Microchip for simultaneous study of absorption, hepatic metabolism and anticancer activity [94].

The institute will develop organs-on-chips models that simulate of radiation damage in the lung, gut, and bone marrow and then use these models to test medical countermeasures for its treatment [103].

In December 2018, a collaborative study by international space station through collaborative research program national center for advancing translational sciences (NCATS) at National Institute of Health (NIH) and center for advancement in sciences in space in partnership with NASA was planned using tissue chips. The aim was to understand the role of microgravity on human health and disease. These chips are expected to behave life astronauts' body experiencing same kind of rapid change [104]. In May 2019, the projects took their first mission and the chips that were studied mimicked lungs, bone marrow, bone and cartilage, kidney and blood brain barrier. In the next year, study of chips mimicking intestine and heart tissues was conducted. In December 2020, a project focusing on the prevention of osteoarthritis after join injuries, muscle wasting and reversal of heart tissue damage was started. In May 2021, the second trip of project started in May 2019, focus was on understanding the kidney stone formation and effect of microgravity on the functioning of kidney was started.

In 2020, the global Organ-on-a-Chip market size is approximately USD 41 million and is expected to reach USD 303.6 million by 2026. In next 7 years, liver segment at a growth rate at a CAGR of 35.4% and kidney segment at a CAGR of 36.1%. Major factors for the growth of organ on a chip technology is increase in collaborations between pharmaceutical companies and organ-on-chip manufacturers for early drug detection and emphasis on the alternatives to animal testing models. Asia-Pacific (China, India and Japan) is expected to witness the highest growth rate and North America to hold largest market share during the forecast period [105].

Some of the major companies developing organ on a chip technology as shown in Table 6 [106]

Technology readiness level, EU Horizon 2020 technology, is a scale that indicates the maturity of a technology and classifies it to different levels.

Peer review publications reports application of this technology in operational environment (Technology Readiness Level - TRL 7) and potential of the technology to be used in preclinical trials [101].

Conclusion and future roadmap

Although, organ on a chip technology has achieved ground breaking success so far yet there is still a long way to go. Apart from the challenges related to surface adsorption effects and poor mixing of fluids in the microfluidic devices, there are several other modification required (Organs on chip review).

Presently, most of the organ on a chip models cover single or combination of few organs. As every individual organ on a chip model is a piece of an entire puzzle, it becomes important to interconnect these models to develop one single chip that mimics all the major organs. Moving towards body on a chip technology, it is important to consider inter-organ scaling, common media and its flow rate and the interdependent functionality of different organs. Although, major organs are simulated using this technology but still there are many other organs such as adipose tissue, retina and placenta to name a few, for which very less studies are done.

In recent studies, a cross talk between microbiome and host metabolism has been highly appreciated. Hence, it becomes important to integrate the microbiome research with organ on a chip technology [106].

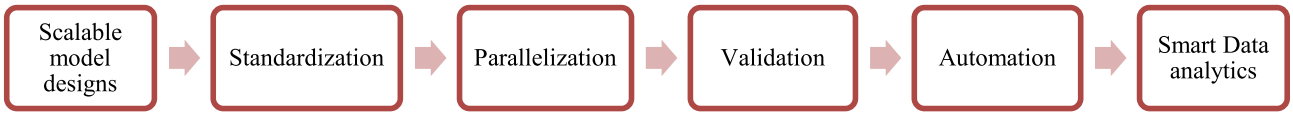

Fig. 6. Important factors for high throughput organ on a chip model development.

Table 4
Methods and materials used for construction of microengineered chips.

Micro-fabrication Methods	Materials	Type of Microarchitectures
Photolithography, Soft lithography, 3D printing, computer numerical code micromilling	Polydimethylsiloxanes (PDMS), Hydrogels, Gelatin methacryloyl, Polyamindes, Polymethylmethacrylate, Polyvinyl chloride	Single layer microfluidic device, 3D compartmentalization, Microfluidic vascular networks

Table 5
Common sources of cells used in organ on a chip technology.

Organs	Cells	References
Lungs	human induced pluripotent stem cells	[99]
Liver	HepG2 cells	[75]
Kidney	mature human podocytesderived from human induced pluripotent stem	[36]
Brain	human induced pluripotent stem cells derived neural cells	[100]
Heart	human induced pluripotent stem cells (iPSCs) – derived 3D cardiac cells	[69]
Gut	Caco 2 cells	[39]
Skin	Induced pluripotent stem cells (iPSC) or commercially available reconstructed skin tissues (EpidermFT, Epiderm, EpiSkin, StratiCELL)	[83]

Table 6
Major companies developing organ on a chips models.

Company	Country	Area
Emulate	U.S.	Lungs-on-chip, gut-on-chip and even blood-brain-barrier-on-chip
Mimetas	Netherlands	Kidney, gut, tumors and many others
Elvesys	France	Microfluidic systems
AxoSim	U.S.	Aims to develop the special microfluidic chips to fight cancer
TaraBiosystems	U.S.	Focuses on developing heart on a chip
Nortis Bio	U.S.	Kidney on a chip
BioIVT	U.S.	Pancreatic islets and the lung airway epithelium are established models
AlveoliX	Switzerland	Human lung on a chip
TissUse	Germany	Multi organ on a chip
BiomimX	Italy	Specialized in the generation of predictive models of human organs and pathologies to test new drugs

Regulatory agencies should also lay guidelines for the validation of organ on a chip technology for variety of potential applications including disease model development [101]. There is a need to transform organ on a chip technology into high throughput organ on a chip technology to expedite the screening process. Parallelization of models, standardized and scalable platform, validation, automation and online data analysis are the important factors shown in Fig. 6 which needs to be incorporated for a high throughput organ on a chip models [107].

Market research shows that North America might hold the largest share of 49% of organ on a chip technology as they might adopt the change from 2D and 3D cell cultures. Pharmaceutical companies, biotechnology companies, academic and research institutions are the major segments to utilize this technology. Increase in research funding across the globe in pharmaceutical/biotechnology sector and growing number of clinical trials based on cell-based therapy will boost the organ on a chip market. [108].

To summarize, organ on a chip technology provides wealth of opportunities including drug toxicity and efficacy studies, *in-vitro* analysis of biochemicals, pathogenesis study of diseases and metabolic activities of human cells.

Declaration of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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